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ORIGINAL INVESTIGATION

Serum cytokine and chemokine changes during Toscana virus meningitis

Jessica Rauch[1] · Lorenzo Zammarchi[2,3] · Giampaolo Corti[2,3] · Alessandro Bartoloni[2,3] · Alexander Schlaphof[1] · Jonas Schmidt-Chanasit[1,4] · Dennis Tappe[1]

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Abstract
Toscana virus is an important arbovirus causing meningitis and meningoencephalitis in countries around the Mediterranean Sea. While the clinical syndrome and laboratory diagnostic procedures have been well described, less is known about the immune response in Toscana virus meningitis and a possible use of cytokine and chemokine changes for the clinical follow-up of patients. We here characterized serum cytokine and chemokine profiles from 37 patients during the acute and convalescent phase of the infection. Only few serum cytokine/chemokine changes were detected during Toscana virus meningitis. Markedly increased concentrations of IP-10, interferon-α, IL-22, and eotaxin were found in the acute phase. Levels of interferon-α, IL-22, and eotaxin remained elevated in the convalescent phase, but decreased concentrations of GM-CSF were detected.

Keywords Toscana virus · Arbovirus · Phlebovirus · Meningitis · Meningoencephalitis · Cytokine

Introduction

Toscana virus (TOSV), an emerging arthropod-borne virus (arbovirus) of the *Phlebovirus* genus, is transmitted to humans by sandflies [1]. TOSV is a leading cause of meningitis and meningoencephalitis in the Mediterranean region [2]. Infections with TOSV are often self-resolving, but may also cause life-threatening disease. The most prevalent symptoms include headache (100%), fever (76–97%), nausea and vomiting (67–88%), and myalgia (18%). In severe cases, meningitis and encephalitis can occur [3–6]. Whereas the clinical syndrome and laboratory diagnostic procedures have been well described, less is known about the immune response in TOSV meningitis and a possible use of cytokine changes for the clinical follow-up of patients. Here, we examined cytokine and chemokine concentrations in the serum of patients with TOSV meningitis during the acute and convalescent phase.

Materials and methods

Serum samples of patients diagnosed with TOSV meningitis at the Infectious and Tropical Diseases Unit, Florence, Italy in the period 1994–2008 were retrieved from an anonymized serum collection stored at – 80 °C. General consent for further studies was obtained at the time of sampling; a dedicated ethical approval was not deemed necessary for this type of study. Thirty-seven acute phase serum samples (first week of onset of symptoms) from 37 patients and eight samples taken during the convalescent phase (day 13–35 after onset of symptoms) from eight patients were available. All patients were admitted for acute meningitis with clear cerebrospinal fluid (CSF). Median age was 37 years (IQR 25–45, range 14–68), male-to-female ratio was 1.2:1. In all cases, TOSV infection was serologically confirmed through detection specific IgM and/or IgG in serum using a commercially available enzyme-linked sorbent assay (Enzywell Toscana virus IgG/IgM ELISA, Diesse Diagnostica,

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✉ Dennis Tappe
tappe@bnitm.de

[1] Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Str. 74, 20359 Hamburg, Germany

[2] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

[3] Infectious and Tropical Diseases Unit, Careggi University Hospital, Florence, Italy

[4] German Centre for Infection Research (DZIF), Partner Site Hamburg-Luebeck-Borstel-Riems, Hamburg, Germany

Monteriggioni, Italy) and immunoblot (recomLine HantaPlus IgG/IgM with recombinant Toscana virus antigen, Mikrogen, Neuried, Germany). In addition, an in-house indirect immunofluorescence antibody test (IFAT, cut off 1:20 [7]) was performed. Acute sera showed IFAT titers of 1:160–1:20,480 (median 1:5120) for IgM and 1:40–1:20,480 (median 1:5120) for IgG, whereas convalescent sera showed IFAT titers of 1:5120–1:10,240 (median 1:10,240) for IgM and 1:2560–1:10,240 (median 1:5120) for IgG. No virus neutralization tests were performed. Serum cytokines and chemokines were analyzed by bead-based LEGENDplex assay (BioLegend, London) from all available samples and 16 healthy controls. Cytokines and chemokines analyzed in our study (with detection limits in parenthesis) were basic fibroblast growth factor (bFGF; 24.17 pg/mL), granulocyte-colony stimulating factor (G-CSF; 12.28 pg/mL), granulocyte macrophage-colony stimulating factor (GM-CSF; 6.67 pg/mL), interferon-α (IFN-α; 1.19 pg/mL), interferon-γ (IFN-γ; 19.53 pg/mL), interleukin (IL)-1β (IL-1β; 4.08 pg/mL), IL-2 (5.49 pg/mL), IL-4 (5.97 pg/mL), IL-5 (5.11 pg/mL), IL-6 (5.49 pg/mL), IL-8 (5.98 pg/mL), IL-9 (4.30 pg/mL), IL-10 (4.58 pg/mL), IL-12p70 (1.31 pg/mL), IL-13 (4.82 pg/mL), IL-17A (4.79 pg/mL), IL-17F (5.13 pg/mL), IL-21 (7.81 pg/mL), IL-22 (41.35 pg/mL), interferon-γ-induced protein-10 (IP-10; N/A), monocyte chemotactic protein-1 (MCP-1; N/A), macrophage inflammatory protein-1α (MIP-1α; 4.95 pg/mL), macrophage inflammatory protein-1β (MIP-1β; 6.06 pg/mL), platelet derived growth factor (PDGF-BB; N/A), regulated on activation, normal T cell expressed and secreted (RANTES; N/A), tumor necrosis factor-α (TNF-α; 4.39 pg/mL), vascular endothelial growth factor (VEGF; 26.92 pg/mL), and eotaxin (N/A).

Results

Serum levels of IFN-α, IP-10, and eotaxin were significantly increased in TOSV meningitis in the acute phase of infection in comparison with healthy controls (Fig. 1). While the concentrations of IFN-α and eotaxin remained significantly elevated also during the convalescent phase, the expression of IP-10 decreased again and was comparable to the controls at that point of time. The levels of IL-22 were markedly increased, however, not significantly, in the acute phase of infection (mean 25.53 pg/mL) and in the convalescent phase (mean 21.98 pg/mL) compared to controls (0.00 pg/mL). In contrast, GM-CSF levels showed significant depressions in the convalescent phase of TOSV meningitis when compared to healthy controls. Serum concentrations of bFGF, G-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10 IL-12p70, IL-13, IL-17A, IL-17F, IL-21, IFN-γ, MCP-1, MIP-1α, MIP-1β, PDGF, RANTES, TNF-α, and VEGF were similar in both patient groups and controls (data not shown).

Discussion

Data on cytokine/chemokine levels during TOSV meningitis are very scarce. In our patients, only a few different cytokines and chemokines in general showed changes during the infection in serum; CSF samples were not available for testing. The samples have been stored for an extended time at – 80 °C and we cannot exclude a possible alteration of cytokine levels, especially between those collected in the 1990s versus those from the following decade.

In our study, increases were seen for IFN-α, IL-22, IP-10, and eotaxin levels, and a decrease was noted for GM-CSF. Previously, normal plasma levels but elevated CSF concentrations in TOSV meningitis have been described for a limited number of mediators tested that encompassed IFN-α, IFN-γ, IL-6, and IL-10 [8]. TNF-α concentrations were comparable to the controls in both plasma and CSF [8]. The cytokine/chemokine elevation profile in CSF during encephalitis caused by other RNA viruses, such as flaviviruses, influenza viruses, and enteroviruses, often imply a Th1 profile with predominant IFN-γ, TNF-α, and IP-10 elevations, but also IL-6 and IL-8 increases [8]. IP-10 which we found elevated in serum, and also eotaxin, are chemokines that recruit immune cells to the area of infection; these include T cells, NK cells, macrophages, and granulocytes [9, 10]. The induction of IL-22, a Th17 profile cytokine which was elevated in the serum of our patients, is organ-specific and antiviral functions for IL-22 and a protective role during central nervous system infections with the subsequent expression of several chemokines have been described previously [11, 12]. The suppression of GM-CSF in the convalescent phase likely reflects a predominant lymphocyte recruitment.

In conclusion, only few serum cytokine/chemokine changes were detected during TOSV meningitis reflecting the often relatively mild clinical aspect of the disease. As a limitation, only a low number of patients could be tested in our investigation. In future studies, more patients and a broad panel of mediators in CSF should be examined to shed more light on the immune responses and resolution of TOSV meningitis.

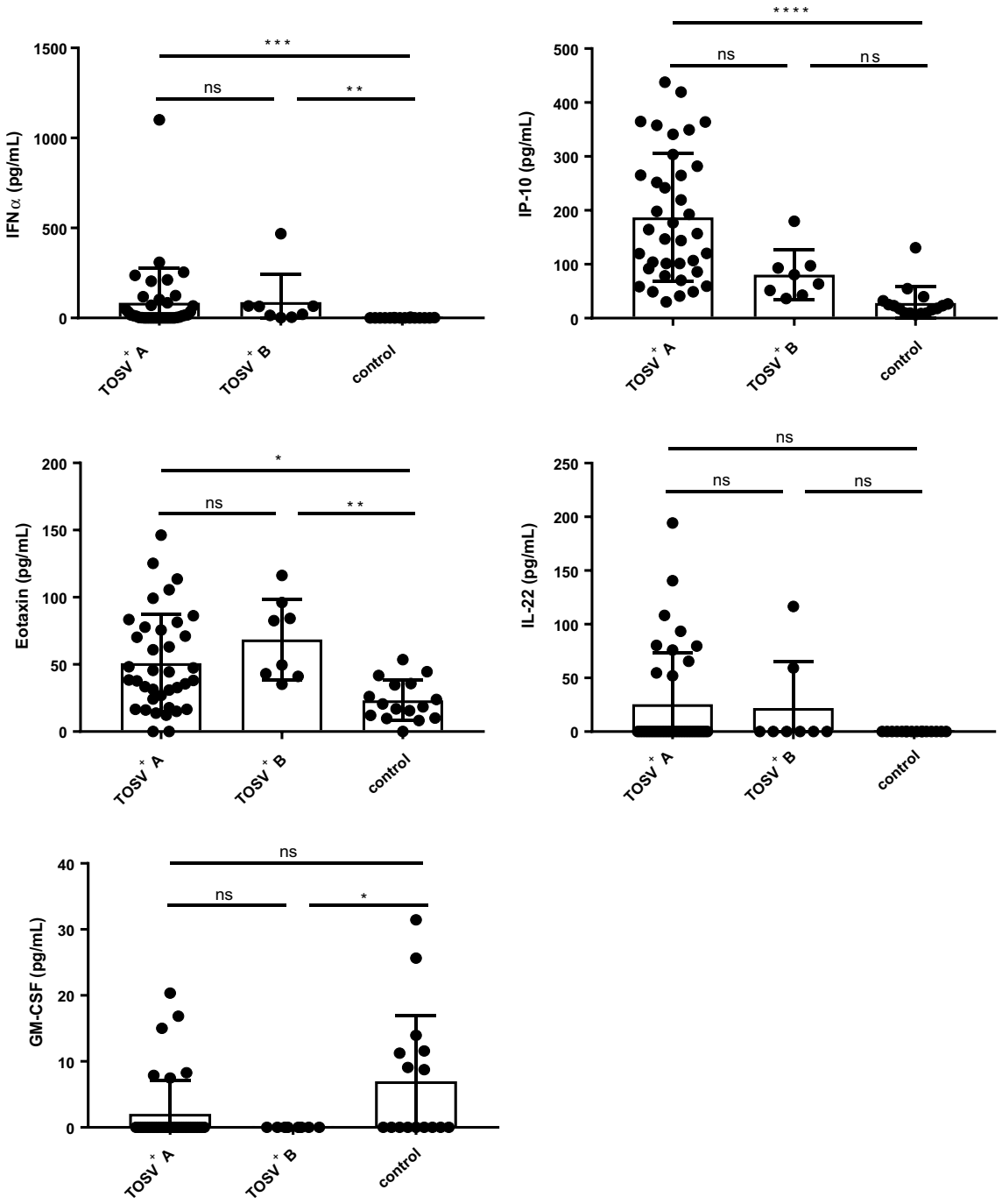

Fig. 1 Serum cytokine and chemokine levels during the acute and convalescent phase of Toscana virus meningitis. 37 sera from patients in the acute phase of Toscana virus meningitis ($TOSV^+$ A), 8 sera from patients in the convalescent phase ($TOSV^+$ B), and 16 sera from healthy controls were analyzed by bead-based LEGENDplex assay. Compared to healthy controls, significant elevations of IFN-α, IP-10, and eotaxin were seen in the acute phase of the infection. Interferon-α and eotaxin were also significantly elevated in the convalescent phase. IL-22 showed marked elevations in both the acute and the convalescent phase. In contrast, GM-CSF was significantly suppressed in the convalescent phase of the infection. Data are expressed as mean ± SD. Statistical analysis was performed with 1-way ANOVA and subsequent Dunn's multiple comparisons test. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). NS, not significant; IFN-α, interferon-α; IP-10, interferon-γ-induced protein-10; GM-CSF, granulocyte macrophage-colony stimulating factor

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Compliance with ethical standards

Conflict of interest All authors declare no conflicts of interest. No author has a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

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